

## Impact of Abdominal Visceral Fat, Growth Hormone, Fitness, and Insulin on Lipids and Lipoproteins in Older Adults<sup>1</sup>

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### **Abstract:**

We examined the relationship between abdominal visceral fat (AVF) and plasma concentrations of lipids and lipoproteins in 19 females (F) (not on estrogen) and 31 males (M) over the age of 60 (age = 66.8 years). In addition, the effects of growth hormone (GH) release, fitness ( $\dot{V}O_2$  peak), insulin, and glucose concentrations (both fasting and in response to an oral glucose tolerance test) on lipids were examined. Subjects were categorized by low (L) and high (H) AVF ( $L < 130 \text{ cm}^2$ ,  $H > 130 \text{ cm}^2$ ), fat mass (FM) (above or below median value), and AVF corrected for fat mass. Factorial analysis of variance (ANOVA) showed that when subjects were divided by AVF and FM, similar results were observed with  $H > L$  ( $P < .05$ ) for very-low-density lipoprotein-cholesterol (VLDL-C), triglycerides (TG), VLDL-TG, apolipoprotein (apo)-B, apo-B VLDL, cholesterol (Chol)/highdensity lipoprotein (HDL), LDL/HDL, apoB/A1 and  $L > H$  for HDL, HDL<sub>2</sub>, HDL<sub>3</sub>, apo A1, and LDL/apo-B LDL. Gender differences were also observed with  $F > M$  for Chol, LDL, HDL, and HDL<sub>2</sub>. When AVF was corrected for FM, these gender differences were still present. After correcting for FM, differences remained between H and L AVF groups for VLDL, TG, VLDL-TG, apo-B, apo-B LDL, apo-B VLDL, apoB/A1 ( $P < .05$ ). Twenty-four hour integrated GH concentration (IGHC) was inversely related to VLDL, TG, VLDL TG, LDL TG, apoB, apoB VLDL, apoB LDL, Chol/HDL, LDL/HDL, and apoB/A1 in F, but not M ( $P < .05$ ).  $\dot{V}O_2$  peak was directly related to Chol, LDL, HDL<sub>3</sub>, and apoB LDL with stronger relationships observed in F. Fasting insulin was related to lipids and lipoproteins in both men and women. These data suggest that, in older adults, elevated levels of AVF, FM, and AVF corrected for FM are associated with unfavorable lipid-lipoprotein profiles and extend similar

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findings reported in younger males and females with elevated AVF. These data also support previous findings indicating that AVF is a primary determinant of GH release.

#### Article:

There is substantial circumstantial evidence to link abdominal visceral fat (AVF) to increased risk for diabetes and coronary artery disease in younger and obese individuals.<sup>1-14</sup> Most of these studies have categorized subjects as having either high or low AVF, with those subjects with high AVF exhibiting increased risk for diabetes and coronary artery disease (CAD).<sup>7-13</sup> However, when reporting a relationship between AVF and risk factors for disease, it is important to control for the concomitant effect of total body fat.

In addition to the confounding effects of total body fat, gender differences have been observed in younger individuals with men having almost twice the amount of AVF for a given body fat mass (FM) as women.<sup>15</sup> After controlling for gender dimorphism in AVF, it was found that most of the differences observed in plasma glucose-insulin homeostasis and in plasma lipoprotein-lipid profiles were no longer significant between men and women.<sup>16</sup> Although few studies have addressed these relationships in older individuals, DiPietro et al<sup>17</sup> recently reported that in healthy older women, elevated AVF was associated with decreased high-density lipoprotein-cholesterol (HDL-C) and an increase in the total/HDL-C ratio.

AVF increases with age in both men and women. This increase is present in normal weight as well as in overweight and obese individuals.<sup>18-20</sup> It has been suggested that the increased cardiovascular risk observed in older adults may be mediated, in part, by increased AVF.<sup>21</sup> However, few data are available that examine the relationship between AVF and serum lipid and lipoprotein profiles (potent risk factors for CAD) in non-obese older adults. Several endocrine changes associated with age may contribute to the observed increase in AVF. These include elevated secretion of corticotropin (ACTH) and cortisol, decreased secretion of sex-specific steroid hormones (particularly in men), and diminished growth hormone (GH) secretion (for a recent review, see Bjorntorp<sup>22</sup>). We have recently reported that in both younger and older individuals the amount of AVF is a primary determinant of 24-hour GH secretion.<sup>23</sup> Others have reported that administration of GH to GH-deficient and obese individuals resulted in a larger relative decrease in AVF than in subcutaneous fat.<sup>24,25</sup> Administration of GH to GH-deficient adults and obese men has also been shown to result in favorable alterations in total cholesterol, low-density lipoprotein-cholesterol (LDL-C), HDL-C, and apolipoprotein (apo)B.<sup>25-27</sup> It has also been suggested that GH may play an important role in lipid metabolism in healthy elderly individuals,<sup>28,29</sup> as well as in GH-deficient adults,<sup>30</sup> and that the neuroregulation of GH secretion is altered in premenopausal women with visceral obesity.<sup>31</sup>

In the present study, we examined the relationship between lipids and lipoproteins and AVF, total body fat, and AVF corrected for total body fat, in older ( $\geq 60$  years) men and women. We hypothesized that similar to observations in younger adults, non-obese older men and women with elevated AVF ( $> 130 \text{ cm}^2$ )<sup>14</sup> (as a result of aging rather than obesity) would exhibit unfavorable lipid and lipoprotein profiles compared with older adults with lower AVF. Based on our previous findings regarding AVF and GH,<sup>23</sup> we further hypothesized that elevated AVF in older men and women would be associated with reduced 24-hour GH release and elevated insulin and glucose concentrations.

## MATERIALS AND METHODS

### *Subjects and Study Protocol*

The study design was approved by the Human Investigation and General Clinical Research Center (GCRC) Advisory Committees of the University of Virginia, and each subject provided written informed consent in accordance with the guidelines established by the University of Virginia Human Investigation Committee. Fifty healthy older (31 men and 19 women; 60 to 79 years old) adults volunteered as subjects. Participants included in this study were recruited by local media advertisement for participation in an ongoing investigation at the GCRC. All subjects completed a detailed medical history and underwent a physical examination before participation. None of the volunteers were taking medications known to affect serum lipids, glucose metabolism, GH secretion, or body composition measures, including exogenous use of estrogen (ie, estrogen replacement therapy) or lipid-lowering drugs. The older women included in this investigation were all postmenopausal. All subjects were nonsmokers and had not undertaken transmeridian travel for at least 4 weeks. All data were collected within a 1-week time frame.

### *Testing Procedures*

*Graded exercise testing.*  $\dot{V}\text{-O}_2$  peak was determined using a graded maximal exercise (treadmill; Quinton Q65, Seattle, WA) test. Subjects were instructed to begin walking on the treadmill at an initial velocity that varied between 60 to 100 ml/min (self-selected). Velocity was increased every 3 minutes by 10 ml/min until the subject reached volitional exhaustion. Metabolic measures were collected during the exercise bout via indirect calorimetry (open circuit spirometry) using a SensorMedics 2900-Z metabolic cart (Yorba Linda, CA).

*AVF.* AVF ( $\text{cm}^2$ ) was determined from a single abdominal computed tomography (CT) scan performed at L4-L5. Scans were performed using a Picker PQ 5000 and analyzed using a newly developed tissue quantification analysis package using a Picker Voxel Q 3D imaging station (Picker International, Cleveland, OH) as described previously.<sup>32</sup> The scanning was performed with 140 kV and a slice thickness of 0.5 cm. Briefly, the subjects were clothed only in a loose gown and examined in a supine position with their arms stretched above their head. An abdominal scan at the level of the L4-L5 inter-vertebral space was performed with no angulation using a lateral pilot for location. AVF cross-sectional areas ( $\text{cm}^2$ ) were calculated by delineating with a mouse computer interface the designated areas and then computing the adipose tissue area using an attenuation range from -190 to -30 Hounsfield units (HU). The measurement boundary for AVF was the internal most aspect of the abdominal and oblique muscle walls and the posterior aspect of the vertebral body [AVF-1<sup>32</sup>]. All CT scan analyses were performed by a single trained investigator (J.L.C.).

*Total body FM and total body percentage fat measurements.* Body density was assessed by hydrostatic weighing (HW), corrected for measured residual lung volume, as previously described.<sup>33</sup> Briefly, subjects were weighed in air on an Accu-weigh beam scale (accurate to 0.1 kg; Metro Equipment, Sunnyvale, CA) and subsequently weighed underwater with a 9-kg Chatillon autopsy scale (accurate to 10 g; Chatillon, New York, NY). Water temperature was maintained between 35°C and 37°C. Underwater weight was measured at residual lung volume for 5 to 10 trials. The average of the last 3 trials was used for body density determination.<sup>33</sup> Residual lung volume was measured on land using an oxygen-dilution technique.<sup>34</sup> The

computational procedure of Siri<sup>35</sup> was used to determine percentage body fat (% fat) from body density measurements. Because of concerns associated with body density determination by HW in the elderly, we examined data on 13 older subjects (7 women, 6 men) who had body density determined on at least 2 occasions. The mean difference between body density measures was 0.009 g/mL, the correlation coefficient was  $r = .97$ , and the total error (TE) was  $\pm 0.0052$  g/mL.<sup>36</sup> In addition, because of reported limitations regarding assumptions underlying the constituents of the FM and fat-free mass (FFM) compartments when using a 2-compartment HW model in older adults, a subset of 33 subjects had their body composition measured using a 4-compartment model.<sup>36</sup>

### *Blood Sampling Procedures*

*GH release.* Twenty-four hour integrated GH concentrations (24-h IGHC; AgL • min) were determined for each subject. The subjects were admitted to the GCRC the evening before blood sampling. The following morning, a venous cannula was inserted into a forearm vein of each arm at 6:30 AM, and blood samples were obtained at 10-minute intervals for 24 hours (from 8 AM on day 1 to 8 AM on day 2) for later measurement of GH. Standardized meals (30% fat, 15% protein, and 55% carbohydrate) were served at 8 AM, 12 noon, and 6 PM. Total daily caloric requirements were determined from basal metabolic rate (BMR) measurements (Delta Trac I, Sensor Medics) performed for 30 minutes (6 to 6:30 AM) and corrected for differences in physical activity patterns by using widely-recognized adjustment factors ranging from 1.3 to 1.5. Volunteers were permitted to ambulate, but were not allowed to nap or sleep until 10 PM during the blood sampling. During the sleeping hours, blood samples were obtained via a 12-ft double lumen catheter kept patent by a heparinized (5,000 UIL) saline solution infused at 30 mL/h using previously described procedures.<sup>37</sup>

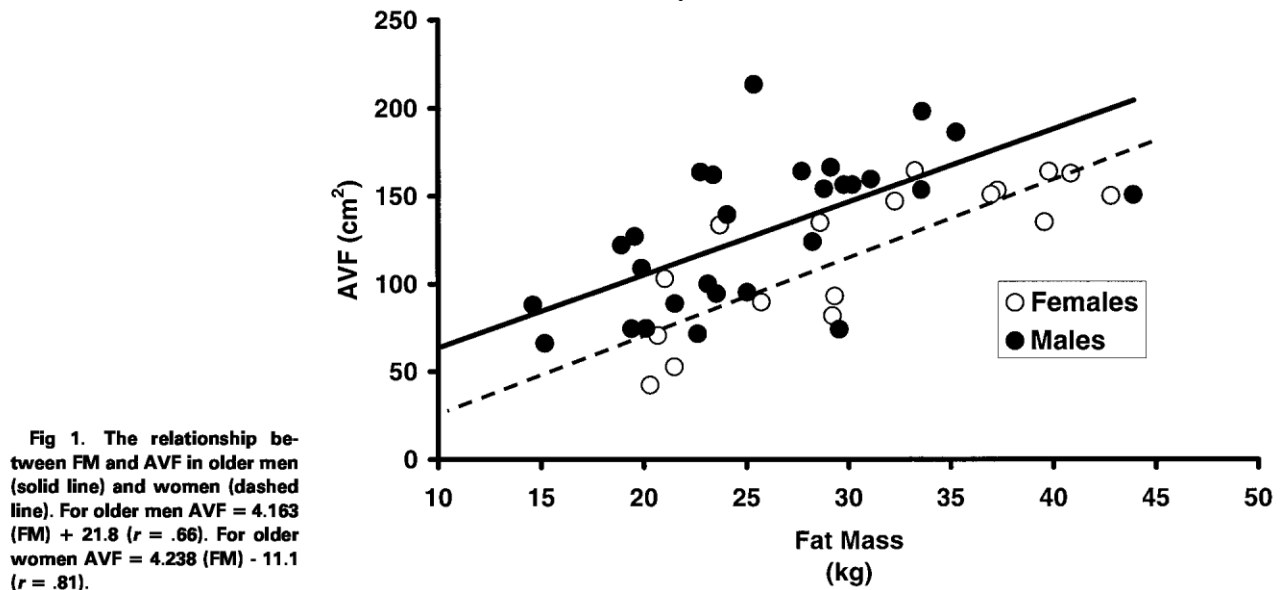
*Fasting insulin, insulin-like growth factor-I, and steroid concentrations.* Insulin and serum insulin-like growth factor (IGF)-I concentrations were determined from blood samples drawn after an overnight fast of 10 to 11 hours at approximately 7 AM during the day of blood sampling for 24-hour GH release. In addition, blood samples were drawn at 6-hour intervals (8 AM, 2 PM, 8 PM, and 2 AM) and pooled for the measurement of estradiol and total testosterone.

*Oral glucose tolerance test.* To assess glucose tolerance, 100 g glucose was administered on a separate day at 8 AM after an overnight fast of 10 to 11 hours. Blood samples for the measurement of glucose and insulin were obtained at -30, 0, 30, 60, 90, 120, 150, and 180 minutes and analyzed using a Beckman (Fullerton, CA) automated glucose analyzer. Glucose area under the curve (AUC) and insulin AUC were determined using the trapezoidal rule.

*Hormonal assays.* GH concentrations in all serum samples were measured using a chemiluminescence assay (Nichols Luma Tag hGH; Nichols Institute Diagnostics, San Juan Capistrano, CA) modified to enhance the sensitivity to 0.002  $\mu$ g/L.<sup>38</sup> This assay detects predominantly the 22-kd form of GH, with 34% cross-reactivity for 20 kd GH (methionylated). The median intra-assay and interassay coefficients of variation were 4.7% and 8.6%, respectively.

Fasting insulin and insulin concentrations during the oral glucose tolerance test (OGTT) were measured in the University of Virginia Diabetes Core Laboratory by a radioimmunoassay (RIA) method with a sensitivity of 11 pmol/L; the interassay CV was 11% at 49 pmol/L and 5.9% at

100 pmol/L. Serum IGF-I concentrations were measured in the University of Virginia GCRC Core Laboratory by RIA after acid- ethanol extraction (Nichols Institute Diagnostics). The median intraassay and interassay CVs were 2.7% and 6.8%, respectively. The pooled E2 and total T concentrations were determined in the University



of Virginia GCRC Core Laboratory using a RIA (Diagnostic Products Corporation, Los Angeles, CA). This assay is known to have 10% cross-reactivity with estrone. Median intra- and interassay coefficients of variation were 3.9% and 9.5% for estradiol, and 6.9% and 10.3% for total testosterone. In addition, hematology, serum chemistries, thyroid function tests, and urinalyses were performed in the University of Virginia Health Sciences Center Clinical Laboratories using routine methods.

**Lipids and lipoproteins.** Blood samples were obtained after an overnight fast of at least 12 hours. Cholesterol (Chol) and triglyceride (TG) levels were determined in plasma and in lipoprotein fractions by enzymatic methods using the Technicon RA-500 analyzer (Bayer Corporation, Tarrytown, NY) as previously described.<sup>39</sup> Plasma very-low-density lipoprotein (VLDL) ( $d < 1.006$  g/mL) were isolated by ultra-centrifugation, and the HDL fraction was obtained after precipitation of LDL in the infranant ( $d > 1.006$  g/mL) with heparin and  $MnCl_2$ .<sup>40</sup> The chol and TG contents of the infranant fraction were measured before and after the precipitation step. The apo A-1 concentration was measured in the infranant and apo B in the plasma and infranant by the rocket immunoelectrophoretic method of Laurell<sup>41</sup> as previously described.<sup>42</sup> The lyophilized serum standards for apo measurements were prepared in the core laboratory at the Lipid Research Center of Laval University Medical Center and calibrated with reference standards obtained from the Centers for Disease Control (Atlanta, GA). The chol content of HDL2 and HDL3 subfractions was also determined after further precipitation of HDL2 with dextran sulfate.<sup>43</sup> Reproducibility of all lipid-lipoprotein measurements has been examined and found to be excellent with all lipid and lipoprotein variables having intraclass correlation coefficients (ICC) above 0.79.<sup>44</sup>

### Statistical Analyses

Subjects were categorized by low (L) and high (H) AVF ( $L < 130 \text{ cm}^2$ ,  $H > 130 \text{ cm}^2$ ; based on data suggesting increased risk for CAD above this threshold<sup>1 4</sup>), FM ( $L < 25 \text{ kg}$  for males,  $< 30 \text{ kg}$  for females;  $H > 25 \text{ kg}$  for males,  $> 30 \text{ kg}$  for females, based on the median values), and AVF corrected for FM. For AVF and FM, 2-way analysis of variance (ANOVA) (Sex X HIL category) was used to analyze the data. To correct AVF for FM, analysis of covariance (ANCOVA) was applied with AVF adjusted for FM. Due to missing data, insulin AUC was not included in these analyses. For each outcome the type I error rate for between group comparisons was adjusted by a least significant difference (LSD) criterion with comparison type I error rate of 0.05.

Correlation analyses were also applied to examine the relationships between the lipid and lipoprotein measures and AVF, FM, 24-h IGHC,  $\text{VO}_2$  peak, IGF-I, fasting insulin, glucose AUC, and insulin AUC.

## RESULTS

Figure 1 shows the relationship between AVF and FM in older men and women. A constant difference in AVF between older men and women was observed at a given level of FM. That is, for any level of total body fat, older men had AVF values that were approximately  $30 \text{ cm}^2$  greater than older women.

Table 1 shows descriptive characteristics for body composition,  $\text{V}\sim\text{O}_2$  peak, fasting insulin, glucose AUC, 24-h IGHC, IGF-I, sex steroids, lipids, and lipoproteins in older females and males categorized by low ( $< 130 \text{ cm}^2$ ) and high ( $> 130 \text{ cm}^2$ ) AVF. When subjects were categorized by AVF, significant main effects were observed for both sex and AVF groups. Older females had higher values than older males for % fat, FM, 24-h IGHC, Chol, LDL Chol, HDL Chol, HDL2 Chol, and LDL/apo B LDL, whereas males had higher values than females for weight, height, AVF,  $\text{V}\sim\text{O}_2$  peak, total testosterone, estradiol, and fasting insulin. When AVF groups were examined, the high AVF group had values that were greater than those observed in the low AVF group for weight, BMI, % fat, FM, AVF, fasting insulin, VLDL Chol, TG, VLDL TG, LDL TG, apo B, apo B VLDL, Chol/HDL, LDL/HDL, and apo B/A1. The low AVF group had higher values for 24-h IGHC, total testosterone, HDL Chol, HDL2 Chol, and LDL/apo B LDL. No significant sex by AVF group interactions were observed, although the sex by AVF interaction for testosterone approached statistical significance ( $P < .07$ ).

Similar results were observed when subjects were divided by high FM ( $> 30 \text{ kg}$  for females and  $> 25 \text{ kg}$  for males) and low FM ( $< 30 \text{ kg}$  for females and  $< 25 \text{ kg}$  for males) (data not shown). When subjects were categorized by FM, significant



**Table 1. ANOVA and ANCOVA Results for Effects of Gender, AVF, and AVF Corrected for FM on Descriptive Characteristics, Body Composition, Fitness, Fasting Insulin, Glucose AUC, 24-h IGHC, IGF-I, Lipids, and Lipoproteins in Older Adults Categorized by Low (<130 cm<sup>2</sup>) and High (>130 cm<sup>2</sup>) AVF**

Variable	Females, Low AVF (n = 8)	Females, High AVF (n = 11)	Males, Low AVF (n = 16)	Males, High AVF (n = 15)	AVF Group	AVF Group, FM Adjusted
Age (yr)	68 (2)	66 (1)	68 (1)	66 (1)	NS	NS
Weight (kg)	62 (2)	78 (3)	81 (3)	91 (2)	M>F; H>L	M>F
Height (cm)	162 (2)	164 (2)	177 (2)	178 (1)	M>F	M>F
BMI	23.6 (0.8)	29.0 (1.0)	25.9 (0.6)	28.8 (0.6)	H>L	M>F
% Fat	39.3 (1.2)	45.9 (1.5)	25.4 (1.1)	32.3 (1.0)	F>M; H>L	F>M
FM (kg)	24.0 (1.4)	35.5 (1.8)	20.7 (1.3)	29.9 (1.4)	F>M; H>L	-----
AVF (cm <sup>2</sup> )	77.4 (7.4)	148.2 (3.7)	87.9 (6.2)	166 (5.0)	M>F; H>L	M>F; H>L
$\dot{V}O_2$ peak (mL/kg/min)	23.1 (1.4)	21.8 (1.2)	31.8 (1.4)	28.8 (1.0)	M>F	M>F
Insulin (pmol/L)	54.7 (5.6)	92.8 (4.6)	70.5 (5.3)	112.3 (11.9)	M>F; H>L	M>F; H>L
24-h GH ( $\mu$ g/L $\times$ min)	1088 (126)	669 (96)	858 (61)	435 (49)	F>M; L>H	F>M; L>H
IGF-I ( $\mu$ g/L)	99.0 (12.6)	122.7 (18.9)	146.4 (12.4)	123.9 (15.1)	NS	NS
Testosterone (nmol/L)	0.6 (0.4)	0.6 (0.4)	16.6 (4.5)	13.2 (3.1)	M>F	M>F
E <sub>2</sub> (pmol/L)	35.9 (16.7)	46.5 (11.3)	82.2 (27.5)	79.3 (31.0)	M>F	M>F
Glucose AUC (mmol/L $\times$ min)	22.7 (1.4)	26.2 (0.9)	25.1 (1.1)	26.1 (1.0)	NS	NS
Cholesterol*	5.36 (0.42)	5.76 (0.28)	4.85 (0.11)	5.08 (0.18)	F>M	F>M
VLDL Chol*	0.31 (0.05)	0.77 (0.19)	0.52 (0.08)	0.75 (0.10)	H>L	NS
LDL Chol*	3.72 (0.38)	3.95 (0.20)	3.47 (0.14)	3.25 (0.09)	F>M	F>M
HDL Chol*	1.34 (0.09)	1.03 (0.05)	1.09 (0.10)	0.85 (0.03)	F>M; L>H	F>M
HDL <sub>2</sub> Chol*	0.75 (0.08)	0.44 (0.06)	0.50 (0.09)	0.34 (0.03)	F>M; L>H	F>M
HDL <sub>3</sub> Chol*	0.59 (0.04)	0.59 (0.04)	0.59 (0.03)	0.51 (0.02)	NS	F>M
Triglycerides*	1.20 (0.10)	2.12 (0.32)	1.43 (0.12)	2.18 (0.26)	H>L	H>L
VLDL TG*	0.58 (0.09)	1.32 (0.28)	0.80 (0.10)	1.48 (0.18)	H>L	H>L
LDL TG*	0.34 (0.03)	0.45 (0.04)	0.36 (0.03)	0.37 (0.04)	H>L	NS
HDL TG*	0.28 (0.03)	0.35 (0.05)	0.27 (0.02)	0.33 (0.06)	NS	NS
Apo B*	0.99 (0.09)	1.18 (0.07)	0.99 (0.05)	1.05 (0.04)	H>L	H>L
Apo B VLDL*	0.06 (0.01)	0.13 (0.02)	0.08 (0.01)	0.12 (0.01)	H>L	H>L
Apo B LDL*	0.93 (0.08)	1.05 (0.06)	0.91 (0.04)	0.94 (0.03)	NS	H>L
Apo A1*	1.37 (0.09)	1.28 (0.04)	1.25 (0.03)	1.22 (0.05)	NS	F>M
Chol/HDL	4.08 (0.31)	5.70 (0.38)	4.98 (0.32)	5.86 (0.32)	H>L	H>L
LDL/HDL	2.85 (0.30)	3.89 (0.23)	3.43 (0.23)	3.90 (0.19)	H>L	NS
LDL/Apo B LDL	3.97 (0.09)	3.78 (0.10)	3.85 (0.09)	3.48 (0.10)	F>M; L>H	F>M
Apo B/A1	0.74 (0.07)	0.93 (0.05)	0.80 (0.04)	0.90 (0.04)	H>L	H>L

NOTE. Data are mean (SE).

Abbreviations: NS, not significant; L, low; H, high.

\*(mmol/L),  $P < .05$  AVF group-2 way ANOVA (Sex  $\times$  AVF);  $P < .05$  AVF group, FM adjusted-2 way ANCOVA (Sex  $\times$  AVF adjusted for FM).

main effects were observed for both sex and FM group. Females had higher values than males for % fat, FM, Chol, LDL Chol, HDL Chol, HDL<sub>2</sub> Chol, apo B, apo B LDL, and apo A1, whereas males had higher values than females for weight, height, BMI, AVF,  $\dot{V}O_2$  peak, total testosterone, estradiol, and fasting insulin. When FM groups were examined, the high FM group had values that were greater than those observed in the low FM group for weight, % fat, FM, AVF, fasting insulin, VLDL Chol, TG, VLDL TG, apo B VLDL, Chol/HDL, LDL/HDL, and apo B/A1. The low FM group had higher values for 24-h IGHC,  $\dot{V}O_2$  peak, HDL Chol, HDL<sub>2</sub> Chol, HDL<sub>3</sub> Chol, apo A1, and LDL/apo B LDL. A significant sex by FM group interaction was observed for body mass index (BMI) with the difference in BMI between the low and high female FM groups being greater than the low and high male FM groups ( $7.5 \nu 2.7 \text{ kg/m}^2$ , respectively). A sex by FM group interaction approached significance for 24-h IGHC and total testosterone ( $P < .07$ ). No other interactions were observed when subjects were grouped by FM. In the subset of 33 subjects who had FM determined with a 4-compartment model of body composition, similar results were observed.

To examine the effects of AVF independent of FM, ANCOVA was run with AVF adjusted for FM. When subjects were categorized by AVF adjusted for FM, significant main effects were observed for both sex and AVF group (Table 1). Females had higher values than males for % fat, 24-h IGHC, Chol, LDL Chol, HDL Chol, HDL<sub>2</sub> Chol, HDL<sub>3</sub> Chol, apo A1, and LDL/apo B LDL, whereas males had higher values than females for weight, height, BMI, AVF, V~O<sub>2</sub> peak, total testosterone, estradiol, and fasting insulin. When AVF adjusted for FM group was examined, the high AVF for FM group had values that were greater than those observed in the low AVF for FM group for AVF, fasting insulin, VLDL Chol, TG, VLDL TG, apo B, apo B VLDL, apo B LDL, Chol/HDL and apo B/A1. The low AVF for FM group had higher values for 24-h IGHC. Significant sex by AVF for FM group interactions were observed for BMI and % fat.

When sex steroid concentrations were examined within gen-

**Table 2. Relationship Between Measures of Lipids and Lipoproteins and Body Composition, Fitness, Insulin, Glucose, Sex Steroids, and 24-h IGHC in Older Females**

Variable	BMI (n = 19)	% Fat (n = 17)	FM (n = 17)	AVF (n = 19)	VO <sub>2</sub> peak (n = 19)	Fasting Insulin (n = 18)	Insulin AUC (n = 12)	Glucose AUC (n = 19)	T (n = 18)	F <sub>2</sub> (n = 18)	24-h IGHC (n = 19)
Cholesterol	-.05	-.05	.06	.10	-.43	.05	.44	-.36	.20	-.10	-.38
VLDL-C	.34	.33	.42	.41	-.20	.54	.15	.10	.47	.24	-.49
LDL-C	-.09	-.12	-.03	.07	-.58	-.08	.39	-.37	.09	-.09	-.27
HDL-C	-.65	-.55	-.70	-.68	.24	-.65	-.18	-.46	-.15	-.43	.36
HDL <sub>2</sub> -C	-.48	-.32	-.48	-.65	-.02	-.62	-.17	-.37	.04	-.51	.39
HDL <sub>3</sub> -C	-.39	-.48	-.45	-.10	-.51	-.12	-.06	-.22	-.37	.11	-.24
Triglycerides	.39	.32	.45	.43	-.25	-.60	-.10	.08	.31	.25	-.51
VLDL TG	.34	.34	.45	.43	-.23	.57	-.10	.12	.30	.25	-.49
LDL TG	.44	.21	.45	.36	-.15	.51	.03	-.09	.15	.12	-.53
HDL TG	.23	.10	.29	.11	-.24	.24	-.21	-.04	.10	.08	-.13
Apo B	.18	.13	.30	.33	-.38	.31	.31	-.18	.20	.06	-.47
Apo B VLDL	.43	.41	.50	.52	-.07	.61	.03	.17	.30	.35	-.47
Apo B LDL	.07	.01	.18	.22	-.46*	.17	.40	-.26	.15	-.01	-.41
Apo A1	-.50	-.49	-.65	-.36	.23	-.37	-.34	-.31	-.01	-.01	.00
Chol/HDL	.50	.41	.59	.58	.05	.59	.13	.07	.29	.30	-.58
LDL/HDL	.46	.34	.54	.54	-.22	.49	.12	.02	.19	.28	-.51
LDL/Apo B LDL	.34	-.28	-.39	-.35	.33	-.60	-.04	-.30	-.11	-.19	.27
Apo B/A1	.49	.33	.57	.51	-.24	.52	-.08	-.04	.20	.06	-.42

NOTE. Correlation coefficients in bold,  $P < .05$ .

Abbreviation: T, testosterone.

der, a significantly higher testosterone concentration was observed within the low AVF males ( $16.6 \pm 4.5$  nmol/L) compared with the high AVF males ( $13.2 \pm 3.1$  nmol/L) ( $P < .02$ ).

To further examine the independent effects of AVF on insulin, 24-h IGHC, and serum lipids and lipoproteins in older adults, we were able to match 7 pairs of male subjects who had similar FM (means = 24.5 kg for both groups), but either low or high AVF (means of  $90.4 \nu 162.2$  cm<sup>2</sup>). With this limited number of subjects, we still observed trends for differences (low  $\nu$  high AVF) in fasting insulin ( $65.5 \nu 98.3$  pmol/L,  $P = .10$ ), 24-h IGHC ( $980 \nu 470$   $\mu$ g/L X min,  $P = .003$ ), testosterone ( $16.7 \nu 12.9$  nmol/L,  $P = .10$ ), TG ( $1.39 \nu 2.08$  mmol/L,  $P = .07$ ), apo B ( $0.95 \nu 1.12$  mmol/L,  $P = .03$ ), apo B LDL ( $0.88 \nu 1.00$  mmol/L,  $P = .07$ ), and apo B/A1 ( $0.81 \nu 0.91$ ,  $P = .10$ ).



The relationship between the lipid and lipoprotein measures and body composition, fitness, insulin, glucose, 24-h IGHC, IGF-I, and sex steroids is shown in Tables 2 (older females) and 3 (older males). When body composition variables were examined, FM and AVF had the strongest relationships with lipids and lipoproteins. The strength of the relationships and the number of significant correlations were greater in women than men. VO<sub>2</sub> peak was related to LDL, HDL<sub>3</sub>, and apoB LDL with stronger relationships observed in women.

Fasting insulin was directly related to VLDL Chol, TG, VLDL TG, LDL TG, HDL TG, apo B VLDL, Chol/HDL, LDL/HDL and apo B/A1 and inversely related to HDL, HDL<sub>2</sub>, and LDL/apo B LDL (Tables 2 and 3). The strength of these relationships and the number of significant correlations were greater in women than men. In contrast, significant relationships between insulin AUC during the OGTT and lipids and lipoproteins were observed only in the men (Table 3), whereas glucose AUC showed little relationship to lipids and lipoproteins.

The association of 24-hour GH release on lipids and lipoproteins was also examined. Inverse relationships were observed between 24-h IGHC and VLDL-C (females), TG (females), VLDL TG (males and females), LDL TG (females), apo B (females), apo B VLDL (males and females), apo B LDL (females), Chol/HDL (females), and LDL/HDL (females) and a direct relationship was observed between 24-h IGHC and LDL/ apo B LDL (males). The strength of the relationships and the number of significant correlations between 24-h IGHC and measures of lipids and lipoproteins was greater in women than men (Tables 2 and 3).

## DISCUSSION

Many studies in younger and obese adults have demonstrated that men and women have, on average, marked differences in regional fat distribution,<sup>15,16</sup> lipoprotein profiles,<sup>6-8,12-14</sup> and indices of glucose-insulin homeostasis.<sup>5,6,9-12,16</sup> Our results in older subjects (age 60 years and over) agree with these previous reports. Older men and women with higher levels of AVF had unfavorable lipid and lipoprotein profiles, higher fasting insulin levels, lower total testosterone levels (particularly in older men), and lower 24-h IGHC (Table 1). Gender differences observed previously in younger adults were also seen in our older subjects. In the present study, older men had higher levels of AVF and fasting insulin levels and lower 24-h IGHC, total cholesterol, LDL chol, HDL-C, HDL<sub>2</sub>-C, HDL<sub>3</sub>-C, apo A1 concentrations, and LDL/apo B LDL in comparison with older women (Table 1). It has been suggested that AVF is a major contributor to the observed gender difference in hepatic lipase activity, although other gender-related differences may have an impact.<sup>45</sup> The higher hepatic lipase activity observed in men is thought to affect both LDL and HDL heterogeneity and may thus be associated with increased CAD risk in men.<sup>45</sup>

**Table 3. Relationship Between Measures of Lipids and Lipoproteins and Body Composition, Fitness, Insulin, Glucose, Sex Steroids, and 24-h IGHC in Older Males**

Variable	BMI (n = 29)	% Fat (n = 27)	FM (n = 27)	AVF (n = 29)	VO <sub>2</sub> peak (n = 29)	Fasting Insulin (n = 28)	Insulin AUC (n = 20)	Glucose AUC (n = 29)	T (n = 28)	E <sub>2</sub> (n = 28)	24-h IGHC (n = 29)
Cholesterol	<b>.51</b>	<b>.41</b>	<b>.43</b>	<b>.26</b>	-.24	-.06	.19	.04	.19	-.13	.08
VLDL-C	-.15	.07	.07	.25	-.19	<b>.42</b>	.29	-.16	-.29	-.41	-.13
LDL-C	.30	.31	.32	.24	-.28	-.26	.09	.04	.32	-.05	.07
HDL-C	<b>-.36</b>	<b>-.43</b>	<b>-.45</b>	<b>-.43</b>	.26	-.26	-.11	.03	.21	.29	.21
HDL <sub>2</sub> -C	<b>-.33</b>	-.34	<b>-.38</b>	<b>-.39</b>	.20	-.25	-.08	.06	.21	.28	.21
HDL <sub>3</sub> -C	-.22	<b>-.40</b>	<b>-.39</b>	-.24	.25	-.15	-.08	-.09	.07	.16	.08
Triglycerides	-.04	.25	.18	<b>.36</b>	-.27	<b>.54</b>	<b>.45</b>	.01	<b>-.40</b>	<b>-.40</b>	-.33
VLDL TG	.00	.27	.22	<b>.43</b>	-.23	<b>.54</b>	.39	-.07	<b>-.37</b>	<b>-.42</b>	<b>-.36</b>
LDL TG	-.20	-.05	-.10	.00	-.16	.25	<b>.43</b>	.23	-.16	-.30	-.17
HDL TG	-.08	.28	.14	.15	-.33	<b>.42</b>	.33	.18	<b>-.49</b>	-.19	-.20
Apo B	-.21	.09	-.11	.16	-.03	.20	<b>.51</b>	.05	-.03	<b>-.40</b>	-.26
Apo B VLDL	-.09	.11	.11	.31	<b>-.36</b>	.30	-.00	-.11	-.28	<b>-.50</b>	<b>-.35</b>
Apo B LDL	-.21	-.15	-.16	.08	.09	.12	<b>.59</b>	.10	.08	-.29	-.18
Apo A1	-.03	-.23	-.17	-.11	.24	-.05	.19	.03	.10	.18	-.07
Chol/HDL	.01	.25	.22	.30	-.18	.23	.11	-.06	-.20	<b>-.42</b>	-.12
LDL/HDL	.06	.23	.22	.26	-.11	.08	.06	-.05	-.07	-.35	-.10
LDL/Apo B LDL	-.11	-.25	-.25	<b>-.45</b>	.22	<b>-.54</b>	<b>-.58</b>	-.10	.35	<b>.37</b>	<b>.35</b>
Apo B/A1	-.08	.13	.09	.26	-.23	.22	.30	.02	-.15	<b>-.52</b>	-.26

NOTE. Correlation coefficients in bold,  $P < .05$ .

Abbreviation: T, testosterone.

Other aspects of the association between body fat distribution and dyslipidemia has been described in detail.<sup>46</sup> Although subjects with high AVF exhibit increased risk for diabetes and CAD,<sup>7-13</sup> when reporting the relationship between AVF and risk factors for disease, it is important to control for the concomitant effect of total body fat. In the present study, unfavorable endocrine and lipoprotein profiles were observed in subjects with higher absolute levels of FM or AVF compared with subjects with lower levels of FM or AVF. In addition, AVF and FM were both significantly correlated with lipid and lipoprotein levels, with stronger relationships observed in older women than in older men (Tables 2 and 3). This makes it difficult to discern the independent effects of AVF and FM on these measures. However, when AVF was corrected for FM, unfavorable lipid and lipoprotein profiles were still observed (Table 1). These data suggest that, similar to observations in young and obese adults,<sup>46</sup> abdominal visceral obesity in older adults is associated with several changes in the metabolic profile predictive for increased risk for metabolic complications. It should be noted that the relationship between AVF and FM appears to be altered in older adults. Although gender differences still exist, they are not as marked as observed in younger adults. That is, in younger adults the slopes of the regression lines relating AVF to FM were greater in men, with men having almost twice the amount of AVF for a given amount of body FM as premenopausal women.<sup>15</sup> In the present study, no gender differences were observed in older adults for the slopes of the regression lines relating AVF to FM. Rather, a constant difference of — 30 cm<sup>2</sup> greater AVF for any given amount of FM was observed in older men compared with the older women (Fig 1).

Elevated TG, apo B, and fasting insulin were among the unfavorable risk factors associated with elevated AVF in the present sample of older adults. These findings have clinical implications for increased risk of CAD in older adults with elevated AVF. The nontraditional risk factors of fasting insulin, apo B levels and small dense LDL size (fasting TG concentrations > 2.0 mmol/L

are associated with an increase in small dense LDL particle size<sup>47</sup>) have been shown to provide more information on CAD risk than the more traditional lipid risk factors of LDL Chol, TG, and HDL Chol.<sup>42</sup> Lamarche et al<sup>47</sup> reported that almost 1 in every 2 ischemic heart disease cases had elevated concentrations of insulin, apo B, and small dense LDL particles, and that this combination of metabolic risk factors resulted in an 18-fold increase in the risk of ischemic heart disease. Furthermore, adjustment for the more traditional cluster of lipid risk factors did not attenuate this relationship. If one examines the traditional lipid risk factors in the present study, one might conclude that elevated AVF does not increase CAD risk in older adults. That is, when AVF was corrected for FM, no significant differences were observed for HDL Chol, and LDL-C between high and low AVF groups (Table 1). However, elevated AVF was associated with higher concentrations of apo B, fasting insulin, and TG. In particular, the mean TG concentration exceeded 2.0 mmol/L in older subjects with elevated AVF (Table 1), suggesting that small dense LDL particles were elevated in these individuals. These data suggest that elevated AVF in older adults is potentially associated with an increased risk of CAD.

Several potential mechanisms have been proposed to explain the relationship between increased AVF and unfavorable lipid and lipoprotein profiles. It has been suggested that the dyslipidemic state associated with visceral obesity results from complex metabolic and hormonal interactions that lead to insulin resistance and increased risk of CAD.<sup>46</sup> Enlarged omental fat cells display a high lipolytic activity that is poorly inhibited by insulin.<sup>3</sup> It has been hypothesized that this contributes to a greater flux of free fatty acids in the portal circulation resulting in reduced hepatic insulin extraction,<sup>48</sup> hyperinsulinemia, increased VLDL synthesis, and apo B production.<sup>3,46</sup> Visceral obesity is also associated with reduced lipoprotein lipase (LPL) levels, which in combination with increased VLDL synthesis, results in elevated plasma concentrations of TG-rich lipoproteins. Higher TG-rich lipoprotein levels are associated with an increased transfer of TG from VLDL to LDL or HDL particles leading to TG enrichment of LDL and HDL.<sup>8</sup> This TG enrichment of LDL and HDL is associated with a reduction in HDL Chol and also provides a good substrate for hepatic TG lipase, ultimately resulting in the generation of small dense LDL particles.<sup>46</sup> The present findings of elevated fasting insulin, VLDL-C, VLDL TG, LDL TG, apo B, and apo B VLDL and reduced HDL-C and HDL2-C in older adults with elevated AVF (Table 1) support this hypothesis.

Several endocrine abnormalities in addition to hyperinsulinemia have been described in individuals with elevated visceral fat.<sup>22</sup> It has been suggested that enhanced sensitivity of the hypothalamo-pituitary-adrenal axis results in increased secretion of corticotropin-releasing hormone, ACTH, cortisol, and in women, adrenal androgens.<sup>22</sup> In addition, individuals with elevated AVF have lower serum GH and sex steroid concentrations.<sup>22,23</sup> The combination of elevated cortisol and insulin concentrations coupled with reduced GH and sex steroids levels observed in individuals with elevated AVF is thought to exert powerful effects leading to increased serum TG concentrations.<sup>22</sup> With aging, serum concentrations of GH, testosterone (in men), and estrogen (in postmenopausal women) decline. These endocrine changes with aging are thought to contribute to the increase in AVF observed with age.<sup>22</sup> Our observations of an inverse relationship between AVF and 24-h IGHC ( $r = -.60$  and  $r = -.65$  in women and men, respectively) coupled with lower 24-h IGHC, lower testosterone levels, and higher fasting insulin concentrations in older individuals with elevated AVF are consistent with reports in younger individuals with the metabolic syndrome. In support of this notion, we recently reported that in

both younger and older individuals AVF was a primary determinant of 24-hour GH release.<sup>23</sup> Furthermore, administration of GH<sup>25</sup> or testosterone<sup>49</sup> to abdominally obese adults results in a reduction in AVF and an improvement in glucose and lipoprotein metabolism.

It has also been suggested that GH may have direct effects on lipid and lipoprotein metabolism.<sup>24-29,50</sup> The proposed mechanism of this direct effect of GH is the ability of GH to induce hepatic LDL receptor expression.<sup>29</sup> This would result in an increased clearance of LDL Cholesterol as well as an increased clearance of VLDL apo B, because the LDL receptor is involved in the hepatic removal of partially depleted VLDL.<sup>29</sup> Thus, it is possible that decreased GH secretion may contribute to the unfavorable lipid profile observed in older adults with elevated AVF.

In summary, results of the present study indicate that in older adults increased FM, AVF, and AVF corrected for FM were related to unfavorable lipid and lipoprotein profiles. In particular, these measures were associated with higher levels of nontraditional risk factors (fasting insulin, TG, and apo B), suggesting a higher risk of CAD. The higher fasting insulin concentrations and reduced 24-h IGHC associated with higher FM and AVF suggest that insulin and GH may be important mediators of the impact of these measures on lipid and lipoprotein metabolism. Sex steroids, particularly testosterone levels in older men, may modulate these relationships.

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